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(54) **IONIC COMPLEXES**

(75) Inventor: **Eric Charles Reynolds**, Carlton (AU)

(73) Assignee: **The University of Melbourne**, Parkville, Victoria (AU)

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None
See application file for complete search history.

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Primary Examiner — Satyanarayana R Gudibande
(74) *Attorney, Agent, or Firm* — Merchant & Gould P.C.

(57) **ABSTRACT**

The present invention provides a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate or amorphous calcium fluoride phosphate complex having a calcium ion greater than about 30 moles of calcium per mole of PP.

18 Claims, 2 Drawing Sheets

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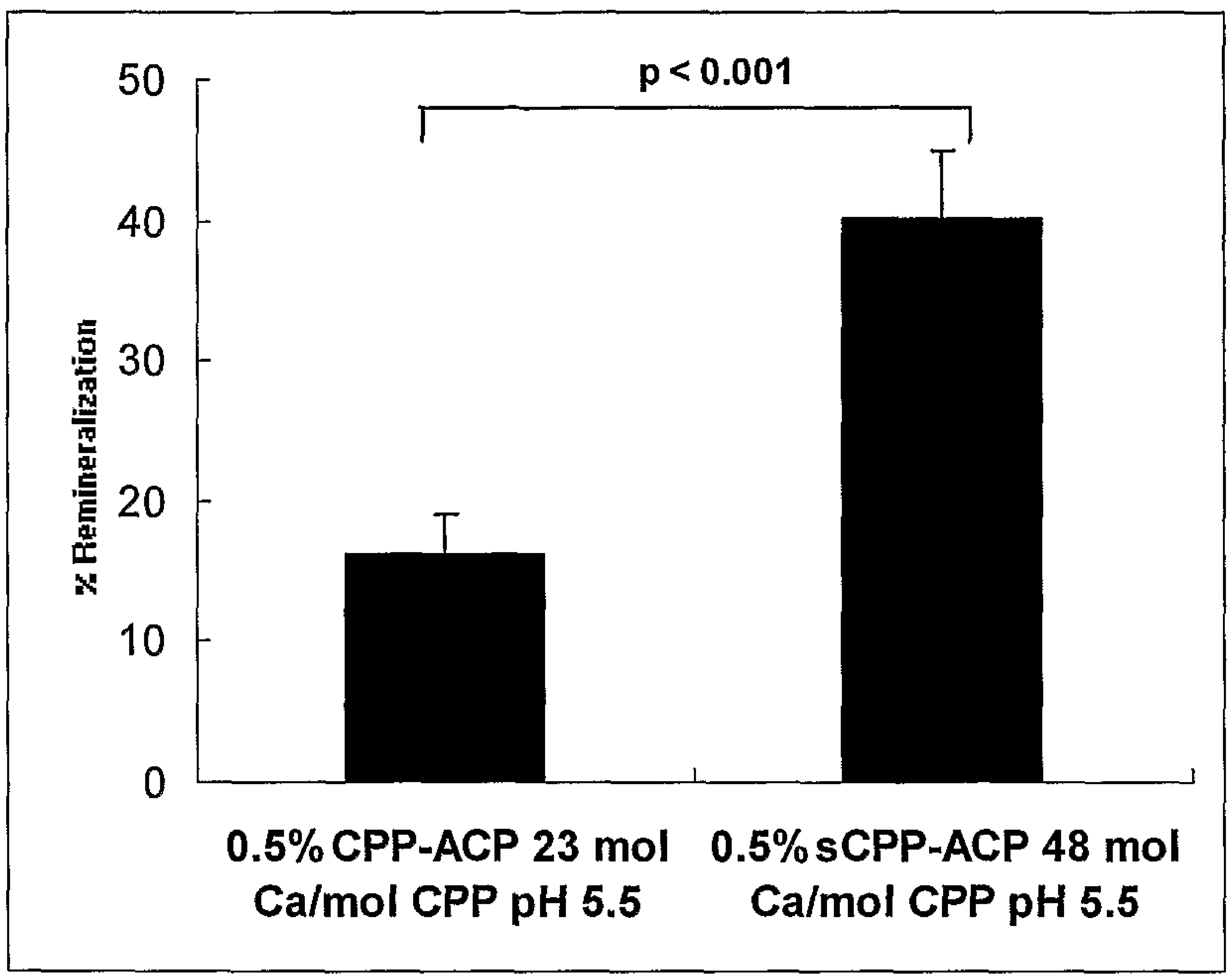
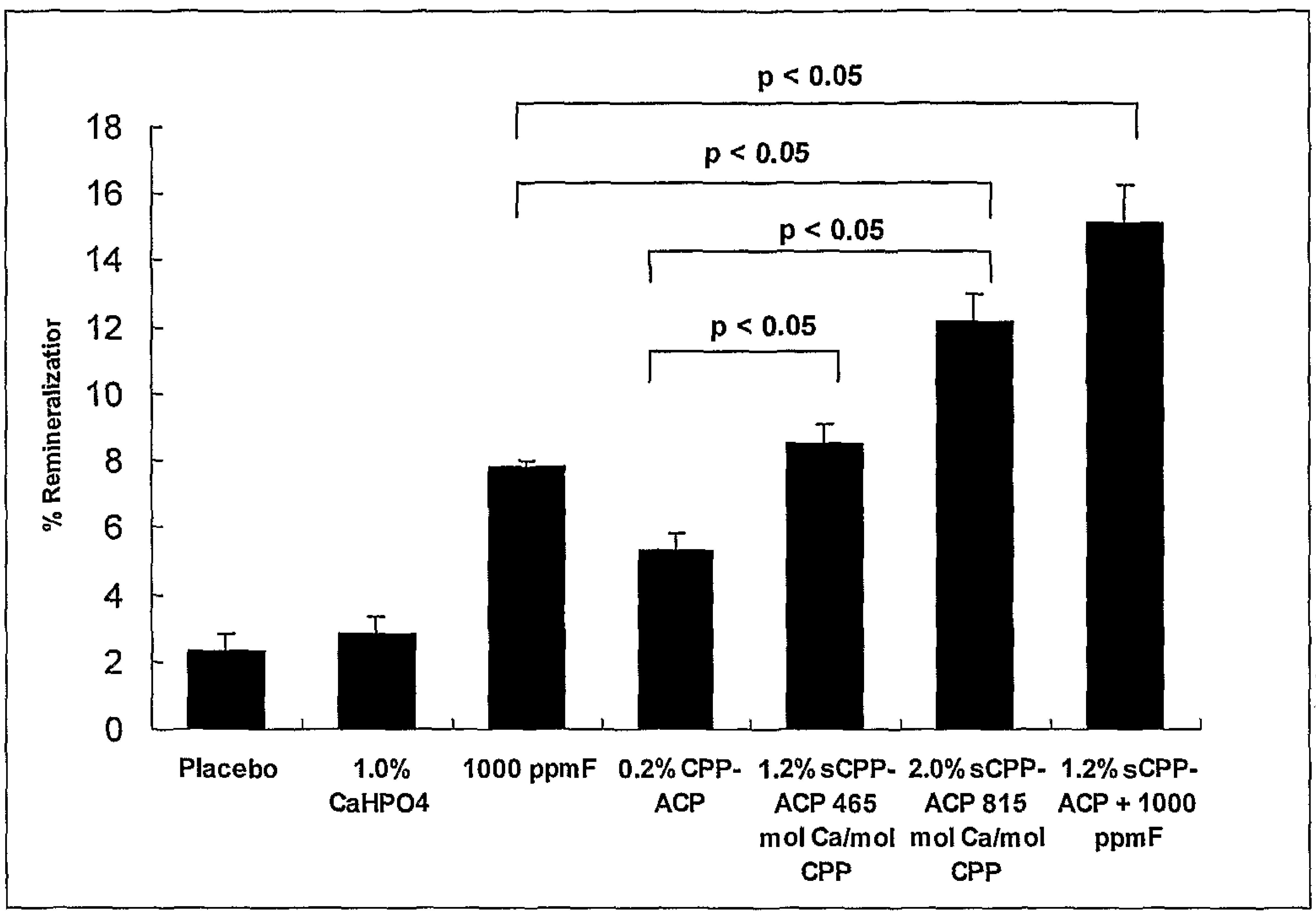
FIGURE 1



p < 0.001
50
40
30
20
10
0
% Remineralization
0.5% CPP-ACP 23 mol Ca/mol CPP pH 5.5
0.5% sCPP-ACP 48 mol Ca/mol CPP pH 5.5

FIGURE 2


p < 0.05
p < 0.05
p < 0.05
p < 0.05
18
16
14
12
10
8
6
4
2
0
% Remineralizatior
Placebo
1.0% CaHPO4
1000 ppmF
0.2% CPP-ACP
1.2% sCPP-ACP 465 mol Ca/mol CPP
2.0% sCPP-ACP 815 mol Ca/mol CPP
1.2% sCPP-ACP + 1000 ppmF

IONIC COMPLEXES

The present invention relates to superloaded complexes of amorphous calcium phosphate and/or amorphous calcium fluoride phosphate stabilised by phosphopeptides/phosphoproteins. These superloaded complexes have anticariogenic properties useful to protect tooth and bone structures as they remineralize (repair) early stages of dental caries as well as other dental/medical applications (including anti-calculus, anti-erosion/corrosion and anti-dentinal hypersensitivity). Methods of making the superloaded complexes of the invention and of treatment or prevention of dental caries, dental calculus, dental erosion/corrosion and dental hypersensitivity are also provided.

BACKGROUND

Dental caries is initiated by the demineralization of hard tissue of the teeth usually by organic acids produced from fermentation of dietary sugar by dental plaque odontopathogenic bacteria. Dental caries is still a major public health problem. Further, restored tooth surfaces can be susceptible to further dental caries around the margins of the restoration. Even though the prevalence of dental caries has decreased through the use of fluoride in most developed countries, the disease remains a major public health problem. Dental erosion/corrosion is the loss of tooth mineral by dietary or regurgitated acids. Dental hypersensitivity is due to exposed dentinal tubules through loss of the protective mineralized layer, cementum and dental calculus is the unwanted accretion of calcium phosphate minerals on the tooth surface. All these conditions, dental caries, dental erosion/corrosion, dental hypersensitivity and dental calculus are therefore imbalances in the level of calcium phosphates. Dental caries, dental erosion/corrosion and dental hypersensitivity can be treated with stabilized amorphous calcium phosphate (ACP) by providing bioavailable calcium and phosphate ions to replace the lost calcium phosphate mineral. Stabilized ACP can also bind to the surface of dental calculus and prevent further accretion. Stabilized ACP and stabilized amorphous calcium fluoride phosphate (ACFP) therefore can play a major role in preventing and treating oral diseases and other medical conditions.

Casein is present in milk in the form of micelles, which are believed to be roughly spherical particles with a radius of about 100 nm, dispersed in a continuous phase of water, salt, lactose and whey proteins. The casein micelles serve as a carrier of calcium phosphate providing a bioavailable source of calcium and phosphate ions for bone and teeth formation. The ability of casein micelles to maintain calcium and phosphate ions in a soluble and bioavailable state is retained by the tryptic multiphosphorylated peptides of the caseins known as the casein phosphopeptides (CPP). WO 98/40406 describes casein phosphopeptide-amorphous calcium phosphate complexes (CPP-ACP) and CPP-stabilised amorphous calcium fluoride phosphate complexes (CPP-ACFP) which have been produced at alkaline pH. Such complexes have been shown to prevent enamel demineralization and promote remineralization of enamel subsurface lesions in animal and human in situ caries models.

The phosphopeptides which are active in forming the complexes do so whether or not they are part of a full-length casein protein. The active casein phosphopeptides (CPP) formed by tryptic digestion have been specified in U.S. Pat. No. 5,015,628 and include peptides Bos α_{s1}-casein X-5P (f59-79) [1], Bos βcasein X-4P (f1-25) [2], Bos α_{s2}-casein X-4P (f46-70) [3] and Bos α_{s2}-casein X-4P (f1-21) [4] as follows:

[1] Gln^{59}-Met-Glu-Ala-Glu-Ser(P)-Ile-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ile-Val-Pro-Asn-Ser(P)-Val-Glu-Gln-Lys^{79} α_{s1}(59-79) (SEQ ID NO: 1)

[2] Arg^{1}-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(P)-Leu-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr-Arg^{25} β(1-25) (SEQ ID NO: 2)

[3] Asn^{46}-Ala-Asn-Glu-Glu-Glu-Tyr-Ser-Ile-Gly-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser(P)-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys^{70} αs2(46-70) (SEQ ID NO: 3)

[4] Lys^{1}-Asn-Thr-Met-Glu-His-Val-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Ile-Ser(P)-Gln-Glu-Thr-Tyr-Lys^{21} αs2(1-21) (SEQ ID NO: 4)

Other casein phosphopeptides that have activity in assisting in the stabilization of superloaded amorphous calcium phosphate complexes are those peptides containing the sequences Ser(P)-Xaa-Glu/Ser(P) where Ser(P) represents a phosphoseryl residue. Therefore the phosphopeptides/phosphoproteins active in stabilizing superloaded amorphous calcium phosphate and amorphous calcium fluoride phosphate complexes are those containing the sequence -A-B-C-, where A is a phosphamino acid, preferably phosphoserine; B is any amino acid including a phosphoamino acid and C is one of the glutamate, aspartate or a phosphoamino acid (SEQ ID NO:5).

Amorphous calcium phosphate stabilized by casein phosphopeptides as described in WO 98/40406 is available commercially in a product sold as Recaldent™ as provided by Recaldent Pty Ltd, Victoria, Australia. However, it would be desirable for an even more effective form of amorphous calcium phosphate stabilized by casein phosphopeptides to be available for treatments. Further, when Recaldent™ is dissolved in a carrier such as distilled water, there is inevitable leakage of ions into the surrounding water to form an equilibrium. This will, in some uses, reduce the calcium phosphate deliverable by the composition, such as for a treatment.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a "superloaded" phosphopeptide or phosphoprotein (PP) stabilized-amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) complex. The complex may be formed at any pH (eg 3-10). Preferably the phosphopeptide includes the sequence -A-B-C-, where A is a phosphoamino acid, preferably phosphoserine, B is any amino acid including a phosphoamino acid and C is glutamic acid, aspartic acid or a phosphoamino acid (SEQ ID NO:5). The phosphoamino acid may be phosphoserine. The PP is superloaded with calcium and phosphate ions. The calcium ions may be in the range 30-1000 mol Ca per mole of PP, or in the range of 30-100 or 30-50 mole Ca per mole of PP. In another embodiment, the mol Ca per mol of PP is at least 25, 30, 35, 40, 45 or 50. The phosphate ions will typically be present in a ratio to the calcium ions (Ca:P) of 1.5-1.8:1. In one embodiment, the ratio is about 1.58:1.

In a further aspect the present invention provides a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP.

In a preferred embodiment, the calcium ion content is in the range of about 30 to 100 moles of calcium per mole of PP.

More preferably, the calcium ion content is in the range of about 30 to about 50 moles of calcium per mole of PP.

The present invention further relates to an aqueous formulation of the PP stabilized ACP or ACFP complex described above.

It will also be understood that the term “comprises” (or its grammatical variants) as used in this specification is equivalent to the term “includes” and may be used interchangeably and should not be taken as excluding the presence of other elements or features.

Surprisingly, the activity of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) as produced using the method described in WO 98/40406, in remineralizing (repairing) enamel subsurface lesions (early stages of tooth decay) can be substantially increased by superloading the casein phosphopeptides with calcium and phosphate ions beyond the amount expected to be possible. The calcium can be in the form of $CaHPO_4$ or calcium lactate and sodium hydrogen phosphate or any other suitable form of calcium salt or phosphate salt.

The PP of the complexes of the present invention may be a casein phosphopeptide (CPP) which may be intact casein or a fragment of casein. The CPP-amorphous calcium phosphate complex formed may be a colloidal complex, where the core particles aggregate to form large (eg 100 nm) colloidal particles suspended in water.

The PP may be from any source; it may be present in the context of a larger polypeptide, including a full length casein polypeptide, or it may be isolated by tryptic or chemical (eg alkaline hydrolysis) digestion of casein or other phosphoamino acid rich proteins such as phosvitin, or by chemical or recombinant synthesis, provided that it comprises the sequence -A-B-C-. The sequence flanking this core sequence may be any sequence. However, those flanking sequences in α_{s1}(59-79) (SEQ ID NO:1), β(1-25) (SEQ ID NO:2), α_{S2}(46-70) (SEQ ID NO:3) and α_{S2}(1-21) (SEQ ID NO:4) are preferred. The flanking sequences may optionally be modified by deletion, addition or conservative substitution of one or more residues. The amino acid composition and sequence of the flanking region are not critical although the preferred flanking regions appear to contribute to the structural action of the motif to maintain the conformation of the peptide so that all phosphoryl and carboxyl groups may interact with calcium ions.

In a preferred embodiment, the PP is selected from the group consisting of α_{s1} (59-79) (SEQ ID NO:1), β(1-25) (SEQ ID NO:2), α_{S2} (46-70) (SEQ ID NO:3) and α_{S2}(1-21) (SEQ ID NO:4).

In a preferred embodiment, at least 40% by weight of the PP in the PP-stabilised ACP or ACFP is a mixture of proteins or protein fragments which are or contain one or more of the peptides (SEQ ID NO: 1) to (SEQ ID NO:4) above. Preferably, at least 60%, more preferably at least 70%, by weight of the PP in the PP-stabilised ACP or ACFP is a mixture of proteins or protein fragments which are or contain the peptides (SEQ ID NO: 1) to (SEQ ID NO:4).

The phosphopeptide is believed to stabilize the superloaded calcium, phosphate (and fluoride) to produce a metastable solution. This binding is believed to inhibit the growth of ACP or ACFP to a size that initiates nucleation and precipitation of calcium phosphate. In this way, calcium and other ions such as fluoride ions can be localised, for instance at a surface on a tooth to prevent demineralization and prevent or reduce formation of dental caries.

Thus, in a further aspect, the invention provides a stable, superloaded ACFP complex or a stable, superloaded ACP complex as described above, which complex acts as a delivery vehicle that co-localises ions including, but not limited to calcium, fluoride and phosphate ions at a target site. In a preferred embodiment, the complex is in a slow-release amorphous form that produces superior anti-caries efficacy. The target site is preferably teeth or bone.

In a further aspect, the invention also provides a method of producing a stable, superloaded complex of ACP or ACFP as described above, comprising the steps of:

(i) obtaining solutions comprising calcium, inorganic phosphate and fluoride (optional); and

(ii) admixing (i) with a solution comprising PP-ACP.

In a preferred embodiment, the PP is casein phosphopeptide (CPP).

In a further aspect of the present invention there is provided a method for increasing the calcium (and phosphate) ion content as ACP/ACFP of a PP stabilized-ACP and/or ACFP including the steps of

(i) obtaining solutions comprising calcium, inorganic phosphate and fluoride (optional); and

(ii) admixing (i) with a solution comprising PP-ACP and/or PP-ACFP.

or

(i) obtaining powders containing calcium eg $CaHPO_4$, calcium lactate, etc. and

(ii) admixing (i) with a PP-ACP and/or PP-ACFP powder.

It has been found that increasing the calcium phosphate loading of PP-ACP complexes in the commercial product known as Recaldent™ may result in a higher viscosity preparation than is suitable for a particular application. Accordingly, it is useful for some applications to prepare the superloaded complexes by dry blending the PP-ACP with calcium phosphate (particularly $CaHPO_4$) for subsequent incorporation into a formulation, for example an oral care formulation such as a toothpaste or chewing gum.

In a further aspect of the present invention there is provided a method for producing a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex having a calcium ion content above about 30 moles of calcium per mole of PP including the steps of:

(i) obtaining a solution including a PP-ACP and/or PP-ACFP complex; and

(ii) mixing with calcium and phosphate ions, while maintaining the solution at a pH of less than 7.

In a further aspect of the present invention there is provided a formulation of a PP stabilized ACP and/or ACFP complex together with at least an equal amount by weight of calcium phosphate. Preferably the calcium phosphate is $CaHPO_4$. Preferably, the calcium phosphate (e.g. $CaHPO_4$) is dry blended with the PP stabilized ACP and/or ACFP complex. In a preferred embodiment, the PP-ACP and/or PP-ACFP complex:calcium phosphate ratio is about 1:1-50, more preferably about 1:1-25, more preferably about 1:5-15. In one embodiment, the PP-ACP and/or PP-ACFP complex:calcium phosphate ratio is about 1:10.

In a further aspect of the present invention there is provided an oral care composition including a formulation of a PP stabilized ACP and/or ACFP complex together with at least an equal amount by weight of calcium phosphate as described above.

In a further aspect of the present invention there is provided a method for producing an oral care formulation that includes a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex having a calcium ion content greater than about 30 moles of calcium per mole of PP when used in the oral cavity including the steps of:

(i) obtaining a powder including a PP-ACP and/or PP-ACFP complex;

(ii) dry blending with an effective amount of calcium phosphate; and

(iii) formulating the dry blended PP-ACP and/or PP-ACFP and calcium phosphate mixture into an oral care formulation.

Preferably, the form of calcium phosphate for dry blending is $CaHPO_4$.

Preferably the oral care formulation is selected from the group consisting of a toothpaste, a tooth crème; a chewing gum; lozenge; a mouthwash and a tooth powder. Without being bound by any theory or mode of action, it is believed that the "superloaded" phosphopeptide is able to deliver a sufficiently high concentration of calcium and phosphate ions, especially ACP and ACFP as the case may be, despite the inherent dilution resulting from the incorporation of the complexes into a physiologically acceptable carrier, and further dilution in, for example, saliva in dental applications. It thus maintains the ionic speciation of the calcium and phosphate ions. The invention is directed to greater amounts of ACP-ACFP at the site of delivery. This can be achieved by a starting material of higher ACP/ACFP content and/or reduced loss or leakage of ACP/ACFP between manufacture and use.

These superloaded complexes are also useful as dietary supplements in subjects who for any reason, such as dietary intolerance, allergy, or religious or cultural factors, are unable or unwilling to consume dairy products in an amount sufficient to supply their dietary calcium requirements. The superloaded complexes of the invention are useful as calcium supplements in subjects in need of stimulation of bone growth, for example subjects undergoing fracture repair, joint replacement, bone grafts, or craniofacial surgery.

In a further aspect of the present invention there is provided a method for remineralizing teeth comprising applying to the teeth a superloaded complex as described above, desirably in a pharmaceutically acceptable carrier. The complex may contain calcium phosphate, calcium fluoride or both. The method is preferably applied to a subject in need of treatment.

In a further aspect, the present invention provides a method for remineralizing a dental surface or subsurface including applying to the dental surface or subsurface a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP.

In a further aspect of the present invention there is provided the use of a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP in the manufacture of a medicament for remineralizing a dental surface or subsurface.

In a further aspect of the present invention there is provided the use of a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP for remineralizing a dental surface or subsurface.

Preferably the dental surface or subsurface is dental enamel, more preferably a surface or subsurface lesion in the dental enamel.

The stable ACFP or ACP superloaded complex may be incorporated into or form in oral care compositions such as toothpaste, mouth washes or formulations for the mouth. This may, for example, aid in the prevention and/or treatment of dental caries or tooth decay. The ACFP or ACP superloaded complex (which may comprise solely CPP-ACP and/or -ACFP complexes, or CPP-ACP and/or -ACFP complexes with calcium phosphate, e.g. $CaHPO_4$) may comprise 0.01-50% by weight of the composition, preferably 0.1%-25%, more preferably 0.5%-20% and optionally 0.5%-10%. For oral compositions, it is preferred that the amount of the CPP-ACP and/or CPP-ACFP administered is 0.01-50% by weight, preferably 0.5%-20% or 0.5%-10% by weight of the composition. In a particularly preferred embodiment, the oral composition of the present invention contains about 1-5% superloaded CPP-ACP (sCPP-ACP). The oral composition of this invention which contains the above-mentioned agents may be prepared and used in various forms applicable to the mouth such as dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs. The oral composition according to this invention may further include additional well known ingredients depending on the type and form of a particular oral composition.

In certain preferred forms of the invention the oral composition may be substantially liquid in character, such as a mouthwash or rinse. In such a preparation the vehicle is typically a water-alcohol mixture desirably including a humectant as described below. Generally, the weight ratio of water to alcohol is in the range of from about 1:1 to about 20:1. The total amount of water-alcohol mixture in this type of preparation is typically in the range of from about 70 to about 99.9% by weight of the preparation. The alcohol is typically ethanol or isopropanol. Ethanol is preferred.

The pH of such liquid and other preparations of the invention is generally in the range of from about 3 to about 10 and typically from about 5.0 to 7.0. The pH can be controlled with acid (e.g. citric acid or benzoic acid) or base (e.g. sodium hydroxide) or buffered (as with sodium citrate, benzoate, carbonate, or bicarbonate, disodium hydrogen phosphate, sodium dihydrogen phosphate, etc).

In one embodiment, the oral composition according to the present invention has a pH of about 5.5.

Accordingly, in a further aspect of the present invention there is provided a composition for remineralizing a dental surface or subsurface including a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP together with a pharmaceutically acceptable carrier and/or excipient.

In a further aspect of the present invention there is provided a composition for remineralizing a dental surface or subsurface consisting essentially of a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP together with a pharmaceutically acceptable carrier and/or excipient.

In another embodiment, the oral composition according to the present invention contains a calcium chelator, eg. pyrophosphate, polyphosphate, citrate, EDTA, etc.

In other desirable forms of this invention, the oral composition may be substantially solid or pasty in character, such as toothpowder, a dental tablet or a toothpaste (dental cream) or gel dentifrice. The vehicle of such solid or pasty oral preparations generally contains dentally acceptable polishing material. Examples of polishing materials are water-insoluble sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated calcium phosphate, anhydrous dicalcium phosphate, calcium pyrophosphate, magnesium orthophosphate, trimagnesium phosphate, calcium carbonate, hydrated alumina, calcined alumina, aluminum silicate, zirconium silicate, silica, bentonite, and mixtures thereof. Other suitable polishing material include the particulate thermosetting resins such as melamine-, phenolic, and urea-formaldehydes, and cross-linked polyepoxides and polyesters. Preferred polishing materials include crystalline silica having particle sizes of up to about 5 microns, a mean particle size of up to about 1.1 microns, and a surface area of up to about 50,000 cm^2/g., silica gel or colloidal silica, and complex amorphous alkali metal aluminosilicate.

When visually clear gels are employed, a polishing agent of colloidal silica, such as those sold under the trademark SYLOID as Syloid 72 and Syloid 74 or under the trademark SANTOCEL as Santocel 100, alkali metal aluminosilicate complexes are particularly useful since they have refractive indices close to the refractive indices of gelling agent-liquid (including water and/or humectant) systems commonly used in dentifrices.

Many of the so-called "water insoluble" polishing materials are anionic in character and also include small amounts of soluble material. Thus, insoluble sodium metaphosphate may be formed in any suitable manner, for example as illustrated by Thorpe's Dictionary of Applied Chemistry, Volume 9, 4th Edition, pp. 510-511. The forms of insoluble sodium metaphosphate known as Madrell's salt and Kurrol's salt are further examples of suitable materials. These metaphosphate salts exhibit only a minute solubility in water, and therefore are commonly referred to as insoluble metaphosphates (IMP). There is present therein a minor amount of soluble phosphate material as impurities, usually a few percent such as up to 4% by weight. The amount of soluble phosphate material, which is believed to include a soluble sodium trimetaphosphate in the case of insoluble metaphosphate, may be reduced or eliminated by washing with water if desired. The insoluble alkali metal metaphosphate is typically employed in powder form of a particle size such that no more than 1% of the material is larger than 37 microns.

The polishing material is generally present in the solid or pasty compositions in weight concentrations of about 10% to about 99%. Preferably, it is present in amounts from about 10% to about 75% in toothpaste, and from about 70% to about 99% in toothpowder. In toothpastes, when the polishing material is silicious in nature, it is generally present in an amount of about 10-30% by weight. Other polishing materials are typically present in amount of about 30-75% by weight.

In a toothpaste, the liquid vehicle may comprise water and humectant typically in an amount ranging from about 10% to about 80% by weight of the preparation. Glycerine, propylene glycol, sorbitol and polypropylene glycol exemplify suitable humectants/carriers. Also advantageous are liquid mixtures of water, glycerine and sorbitol. In clear gels where the refractive index is an important consideration, about 2.5-30% w/w of water, 0 to about 70% w/w of glycerine and about 20-80% w/w of sorbitol are preferably employed.

Toothpaste, creams and gels typically contain a natural or synthetic thickener or gelling agent in proportions of about 0.1 to about 10, preferably about 0.5 to about 5% w/w. A suitable thickener is synthetic hectorite, a synthetic colloidal magnesium alkali metal silicate complex clay available for example as Laponite (e.g. CP, SP 2002, ID) marketed by Laporte Industries Limited. Laponite D is, approximately by weight 58.00% SiO_2, 25.40% MgO, 3.05% Na_2O, 0.98% Li_2O, and some water and trace metals. Its true specific gravity is 2.53 and it has an apparent bulk density of 1.0 g/ml at 8% moisture.

Other suitable thickeners include Irish moss, iota carrageenan, gum tragacanth, starch, polyvinylpyrrolidone, hydroxyethylpropylcellulose, hydroxybutyl methyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose (e.g. available as Natrosol), sodium carboxymethyl cellulose, and colloidal silica such as finely ground Syloid (e.g. 244). Solubilizing agents may also be included such as humectant polyols such propylene glycol, dipropylene glycol and hexylene glycol, cellosolves such as methyl cellosolve and ethyl cellosolve, vegetable oils and waxes containing at least about 12 carbons in a straight chain such as olive oil, castor oil and petrolatum and esters such as amyl acetate, ethyl acetate and benzyl benzoate.

It will be understood that, as is conventional, the oral preparations are to be sold or otherwise distributed in suitable labelled packages. Thus, a jar of mouthrinse will have a label describing it, in substance, as a mouthrinse or mouthwash and having directions for its use; and a toothpaste, cream or gel will usually be in a collapsible tube, typically aluminium, lined lead or plastic, or other squeeze, pump or pressurized dispenser for metering out the contents, having a label describing it, in substance, as a toothpaste, gel or dental cream.

Organic surface-active agents may be used in the compositions of the present invention to achieve increased prophylactic action, assist in achieving thorough and complete dispersion of the active agent throughout the oral cavity, and render the instant compositions more cosmetically acceptable. The organic surface-active material is preferably anionic, nonionic or ampholytic in nature and preferably does not interact with the active agent. It is preferred to employ as the surface-active agent a detersive material which imparts to the composition detersive and foaming properties. Suitable examples of anionic surfactants are water-soluble salts of higher fatty acid monoglyceride monosulfates, such as the sodium salt of the monosulfated monoglyceride of hydrogenated coconut oil fatty acids, higher alkyl sulfates such as sodium lauryl sulfate, alkyl aryl sulfonates such as sodium dodecyl benzene sulfonate, higher alkylsulfo-acetates, higher fatty acid esters of 1,2-dihydroxy propane sulfonate, and the substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds, such as those having 12 to 16 carbons in the fatty acid, alkyl or acyl radicals, and the like. Examples of the last mentioned amides are N-lauroyl sarcosine, and the sodium, potassium, and ethanolamine salts of N-lauroyl, N-myristoyl, or N-palmitoyl sarcosine which should be substantially free from soap or similar higher fatty acid material. The use of these sarconite compounds in the oral compositions of the present invention is particularly advantageous since these materials exhibit a prolonged marked effect in the inhibition of acid formation in the oral cavity due to carbohydrates breakdown in addition to exerting some reduction in the solubility of tooth enamel in acid solutions. Examples of water-soluble nonionic surfactants suitable for use are condensation products of ethylene oxide with various reactive hydrogen-containing compounds reactive therewith having long hydrophobic chains (e.g. aliphatic chains of about 12 to 20 carbon atoms), which condensation products ("ethoxamers") contain hydrophilic polyoxyethylene moieties, such as condensation products of poly (ethylene oxide) with fatty acids, fatty alcohols, fatty amides, polyhydric alcohols (e.g. sorbitan monostearate) and polypropyleneoxide (e.g. Pluronic materials).

The surface active agent is typically present in amount of about 0.1-5% by weight. It is noteworthy, that the surface active agent may assist in the dissolving of the active agent of the invention and thereby diminish the amount of solubilizing humectant needed.

Various other materials may be incorporated in the oral preparations of this invention such as whitening agents, preservatives, silicones, chlorophyll compounds and/or ammoniated material such as urea, diammonium phosphate, and mixtures thereof. These adjuvants, where present, are incorporated in the preparations in amounts which do not substantially adversely affect the properties and characteristics desired.

Any suitable flavouring or sweetening material may also be employed. Examples of suitable flavouring constituents are flavouring oils, e.g. oil of spearmint, peppermint, wintergreen, sassafras, clove, sage, eucalyptus, marjoram, cinnamon, lemon, and orange, and methyl salicylate. Suitable sweetening agents include sucrose, lactose, maltose, sorbitol, xylitol, sodium cyclamate, perillartine, AMP (aspartyl phenyl alanine, methyl ester), saccharine, and the like. Suitably, flavour and sweetening agents may each or together comprise from about 0.1% to 5% more of the preparation.

The invention also provides use of a composition as described above. In the preferred practice of this invention an oral composition according to this invention such as mouthwash or dentifrice containing the composition of the present invention is preferably applied regularly to the gums and teeth, such as every day or every second or third day or preferably from 1 to 3 times daily, at a pH, preferably of about 3.0 to about 10.0 or more preferably 5.0 to about 9.0, for at least 2 weeks up to 8 weeks or more up to a lifetime. In one embodiment, the pH of the oral composition is about 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0.

The compositions of this invention can also be incorporated in lozenges, or in chewing gum or other products, e.g. by stirring into a warm gum base or coating the outer surface of a gum base, illustrative of which are jelutong, rubber latex, vinylite resins, etc., desirably with conventional plasticizers or softeners, sugar or other sweeteners or such as glucose, sorbitol and the like.

In another embodiment, the complex of the invention is formulated to form a dietary supplement preferably comprising 0.1-100% w/w, more preferably 1-50% w/w, most preferably 1-10% and particularly 2% w/w of a comestible. The complex may also be incorporated into food products.

In a further aspect, the invention provides compositions including pharmaceutical compositions comprising any of the superloaded ACFP and/or ACP complexes as described above together with a pharmaceutically-acceptable carrier. Such compositions may be selected from the group consisting of dental, anticariogenic compositions, therapeutic compositions and dietary supplements. Dental compositions or therapeutic compositions may be in the form of a gel, liquid, solid, powder, cream or lozenge. Therapeutic compositions may also be in the form of tablets or capsules. In one embodiment, the superloaded ACP and/or ACFP complexes are substantially the only remineralizing active components of such a composition. In a further embodiment the superloaded ACP and/or ACFP complexes form after the composition, as an oral care composition, is contacted with saliva in the oral cavity.

In a further aspect, there is provided a method of treating or preventing dental caries or tooth decay, dental erosion/corrosion, dentinal hypersensitivity and dental calculus comprising the step of administering a complex or composition of the invention to the teeth or gums of a subject in need of such treatments. Topical administration of the complex is preferred.

According to a further aspect of the invention there is provided a composition for dental restoration, including a dental restorative material to which has been added a superloaded ACFP and/or ACP complex according to the present invention. The base of the dental restorative material can be a glass ionomer cement, a composite material or any other restorative material which is compatible. It is preferred that the amount of superloaded CPP-ACP complex or superloaded CPP-ACFP complex included in the dental restorative material is 0.01-80% by weight, preferably 0.5-10% and more preferably 1-5% by weight. The dental restorative material of this invention which contains the above mentioned agents may be prepared and used in various forms applicable to dental practice. The dental restorative material according to this invention may further include other ions, eg. antibacterial ions Zn^{2+}, Ag^{+}, etc or other additional ingredients depending on the type and form of a particular dental restorative material. It is preferable that the pH of the superloaded CPP-ACP complex or superloaded CPP-ACFP complex be between 2-10, more preferably 5-9 and even more preferably 5-7. It is preferable that the pH of the dental restorative material containing the superloaded CPP-ACP complex or superloaded ACFP complex be between 2-10, more preferably 5-9 and even more preferably 5-7.

The invention is also directed to a method of manufacture of a restorative composition. Preferably, the method includes the addition of a superloaded ACP and/or ACFP complex as described above, to a base dental restorative material. The invention also relates to use of a restorative composition as stated above for the treatment and/or prevention of dental caries.

In a further aspect of the present invention there is provided a kit for use in the preparation of a composition for dental restoration including (a) dental restorative material and (b) a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP. The kit may optionally include instructions for use for the preparation of a composition for dental restoration.

In a preferred embodiment, the dental restorative material is a porous dental cement. In a further preferred embodiment, the dental restorative material is a glass ionomer cement. Without being bound by any theory or mode of action, it is believed that micropores in certain types of porous dental cement, such as glass ionomer cements, allow the passage of the complexes of the present invention to the dental surface to promote remineralization of the dental material.

The invention also relates to a kit for use in the preparation of a composition for dental restoration including (a) dental restorative material and (b) superloaded CPP-ACP complex and/or superloaded CPP-ACFP complex. The kit may optionally include instructions for use for the preparation of a composition for dental restoration.

The invention also relates to a kit for use in the preparation of a composition for dental restoration including (a) dental restorative material (b) casein phosphopeptide (c) calcium ions and (d) phosphate ions, and optionally fluoride ions. The kit may optionally include instructions for use for the preparation of a composition for dental restoration.

The invention also provides a method of treatment and/or prevention of dental caries, dental erosion/corrosion, dental hypersensitivity and dental calculus in animals including humans including providing the composition according to the invention, or manufactured according to the invention, and applying to teeth in an animal in need of treatment and/or prevention.

In a further aspect, the invention relates to methods of treating one or more conditions related to calcium loss from the body, especially from the bones, calcium deficiency, calcium malabsorption, or the like. Examples of such conditions include, but are not limited to, osteoporosis and osteomalacia. In general any condition which can be improved by increased calcium bioavailability is contemplated.

In a further aspect of the present invention there is provided the use of a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP in the manufacture of a composition for the treatment and/or prevention of dental caries, dental erosion/corrosion, dental hypersensitivity, and/or dental calculus.

In a further aspect of the present invention there is provided the use of a composition including a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP for the treatment and/or prevention of dental caries, dental erosion/corrosion, dental hypersensitivity, or dental calculus.

It will be clearly understood that, although this specification refers specifically to applications in humans, the invention is also useful for veterinary purposes. Thus in all aspects the invention is useful for domestic animals such as cattle, sheep, horses and poultry; for companion animals such as cats and dogs; and for zoo animals.

In the figures:

FIG. **1**: Enhanced remineralization of enamel subsurface lesions in vitro by CPP superloaded with calcium and phosphate ions.

FIG. **2**. Enhanced remineralization of enamel subsurface lesions in situ by toothpaste containing CPP superloaded with calcium phosphate.

The invention will now be described with reference to the following non-limiting examples.

EXAMPLE 1

Recaldent™ (CPP-ACP) was purchased from Recaldent Pty Ltd, Victoria, Australia. The product (#841117) contained 14.3% calcium, 22.3% phosphate and 47% casein phosphopeptide on a weight basis. The product was dissolved at 0.5% and adjusted to pH 5.5 by the addition of HCl. Calcium and phosphate ions were then added by titrating 3.25 M $CaCl_2$ and 2M NaH_2PO_4 while keeping the pH at 5.5 with the addition of 2.5 M NaOH. The titration of calcium and phosphate ions was continued until the solution became translucent. The concentration of calcium and phosphate added was recorded. The solution may also be formed by titrating calcium and phosphate ions into a 0.5% CPP-ACP solution and letting the pH fall to 5.5 by the addition of further calcium phosphate.

TABLE 1

Calcium and phosphate levels of normal and superloaded CPP-ACP

	Calcium		Phosphate	
	mmol/L	mol/mol CPP	mmol/L	mol/mol CPP
Normal 0.5% w/v CPP-ACP	17.8	22.8	11.6	14.8
Superloaded 0.5 w/v CPP-ACP (sCPP-ACP)	37.8	48.3	23.6	30.2

These results demonstrate that CPP-ACP can be superloaded with calcium and phosphate ions to produce thermodynamically stable complexes in a metastable solution.

EXAMPLE 2

In another example Recaldent™ (CPP-ACP) powder was dry blended with $CaHPO_4$ powder in the ratio CPP-ACP: $CaHPO_4$ equals 1:10 on a weight basis. This powder was then added to sugar-free gum and toothpaste formulations at 1-5% w/w.

EXAMPLE 3

Comparison of Remineralization of Enamel Subsurface Lesions In Vitro by Normal CPP-ACP and Superloaded CPP-ACP (sCPP-ACP)

The polished enamel surface of extracted human third molars were sawn as a slab (8×4 mm^2) and covered with acid resistant nail varnish to form an occlusal-half and a gingival-half mesiodistal window (1×7 mm^2) separated by 1 mm [Reynolds E. C. (1997) *J. Dent. Res.* 76, 1587-1595]. Subsurface enamel lesions were created in these windows using the Carbopol method of White [White D. J. (1987) *Caries Res* 21, 228-242] as modified by Reynolds [Reynolds E. C. (1997) *J. Dent. Res.* 76, 1587-1595]. The enamel slabs were sawn in half to 4×4 mm^2 blocks. The gingival-half lesion on one block and the occlusal-half lesion on the other block were sealed with varnish to create the demineralized controls as described by Reynolds [Reynolds E. C. (1997) *J. Dent. Res.* 76, 1587-1595].

The enamel half-lesions were exposed to the two different remineralization solutions for 10 days at 37° C. without mixing. The remineralization solutions were 0.5% w/v CPP-ACP adjusted to pH 5.5 with HCl and superloaded CPP-ACP prepared in Example 1.

After remineralization each pair of blocks was dehydrated in ethanol and embedded in methyl-methacrylate resin (Paladur, Kulzer, Germany). Three 200-300 μm sections were cut perpendicular to the lesion surface, lapped down to 80±5 μm and radiographed beside an aluminium stepwedge of 10×14 μm thick increments as described previously.

Radiographic images of the lesions were viewed via transmitted light through a Dilux 22 microscope (Ernst Leitz Wetzlar, Germany). The images were acquired by video camera (Sony DXC 930P) and digitized (Scion imaging corporation, colour grabber **7**) under the control of imaging software (Optimas 6.2). Images of the lesions, controls and the aluminium stepwedge were scanned as previously described by Shen et al. [Shen P. et al., (2001) *J. Dent. Res.* 80, 2066-2070]. The enamel section thickness was measured and volume percentage mineral data determined using the equation of Angmar [Angmar B. et al., (1963) *Ultrastructural Res* 8, 12-23] as previously described by Shen at al. [Shen P. et al., (2001) *J. Dent. Res.* 80, 2066-2070]. The percentage remineralization (% R) was also calculated as previously described by Shen et al. [Shen P. et al., (2001) *J. Dent. Res.* 80, 2066-2070].

The remineralization of the enamel subsurface lesions is shown in FIG. **1**.

The results of FIG. **1** demonstrate that a superloaded CPP-ACP (sCPP-ACP) solution is superior to a normal CPP-ACP solution in remineralization of enamel subsurface lesions in vitro.

EXAMPLE 4

The ability of toothpaste formulations containing superloaded CPP-ACP (sCPP-ACP) to remineralize enamel subsurface lesions was investigated in a randomized, cross-over, double-blind in situ clinical study using the protocol of Reynolds et al. [Reynolds E. C. et al., (2003) *J Dent Res.* 82, 206-211]. Ten subjects wore removable palatal appliances with six, human enamel, half-slabs containing sub-surface demineralized lesions prepared as described in Example 3. The other half of each enamel slab was stored in a humidified container and was used as the control demineralized lesion. There were seven treatments in the study, toothpaste B containing 0.2% w/w normal CPP-ACP, toothpaste C containing 0.2% CPP-ACP/1.0% $CaHPO_4$ (referred to in FIG. **2** and Example 5 as "1.2% sCPP-ACP"), toothpaste E containing 1.0% $CaHPO_4$, toothpaste F containing 1000 ppm F, toothpaste G containing 1.2% sCPP-ACP plus 1000 ppmF, toothpaste D containing 0.2% CPP-ACP/1.8% $CaHPO_4$ (referred to in FIG. **2** and Example 5 as "2.0% sCPP-ACP"), and a control toothpaste A (placebo). The pastes were used for 30 s periods, four times per day. The appliances were worn while using the paste and then for 1 hr after using the paste. Each treatment was for 14 days duration and each of the ten subjects carried out each treatment with a one week rest between the treatments. At the completion of each treatment the enamel slabs were removed, paired with their respective demineralized control, embedded, sectioned and subjected to microradiography and computer-assisted densitometric image analysis to determine the level of remineralization. The results presented as percentage enamel remineralization (% R) are shown in FIG. **2** and demonstrate that 0.2% CPP-ACP superloaded with either 1.0% $CaHPO_4$ (1.2% sCPP-ACP) or 1.8% $CaHPO_4$ (2.0% sCPP-ACP) remineralizes enamel subsurface lesions significantly better than the normal 0.2% CPP-ACP or the $CaHPO_4$ alone at the same concentration. The 2.0% sCPP-ACP paste was significantly better than the paste containing 1000 ppm fluoride. Further, 1.2% sCPP-ACP plus 1000 ppm F showed an additive effect over 1.2% sCPP-ACP or 1000 ppm F alone.

EXAMPLE 5

Toothpaste Formulations Containing Superloaded CPP-ACP (sCPP-ACP)

Formulation 1

Ingredient	% w/v 1	% w/v 2	% w/v 3
Sorbitol	53.0	53.0	53.0
Silica (Zeodent 119)	20.0	20.0	20.0
Purified water	balance	balance	balance
Sodium lauryl sulphate	4.0	4.0	4.0
sCPP-ACP	1.2	1.2	2.0
Sodium monofluorophosphate	0.3	—	—
Flavour	1.0	1.0	1.0
Sodium carboxymethyl cellulose	0.75	0.75	0.75
Titanium dioxide	0.525	0.525	0.525
Xanthan gum	0.475	0.475	0.475
Sodium saccharin	0.350	0.350	0.350

pH adjusted to 7.0 with phosphoric acid

Formulation 2

Ingredient	% w/v	% w/v	% w/v
Sorbitol	22.0	22.0	22.0
Irish Moss	1.0	1.0	1.0
Gantrez	19.0	19.0	19.0
Purified water	balance	balance	balance
Sodium monofluorophosphate	—	—	0.76
Sodium saccharine	0.3	0.3	0.3
Pyrophosphate	2.0	2.0	2.0
Hydrated alumina	47.0	47.0	47.0
Flavour	0.95	0.95	0.95
sCPP-ACP	1.0	2.0	2.0
Sodium lauryl sulphate	2.0	2.0	2.0

pH adjusted to 5-7 with NaOH

Formulation 3

Ingredient	% w/v
Dicalcium phosphate dihydrate	45.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Sodium lauryl sulphate	1.5
Sodium lauryl sarconisate	0.5
Flavour	1.0
Sodium saccharine	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Dextranase	0.01
sCPP-ACP	5.0
Purified water	balance

pH adjusted to 5-7 with phosphoric acid

Formulation 4

Ingredient	% w/v
Sorbitol	22.0
Irish moss	1.0
Gantrez	19.0
Purified water	balance
Sodium saccharin	0.3
Pyrophosphate	2.0
Hydrated alumina	43.0
Sodium monofluorophosphate	0.3
Flavour	0.95
sCPP-ACP	5.0
Sodium lauryl sulphate	2.0

pH adjusted to 5.5 with NaOH

Formulation 5

Ingredient	% w/v
Dicalcium phosphate dihydrate	45.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Sodium lauryl sulphate	1.5
Sodium lauryl sarconisate	0.5
Flavour	1.0
Sodium saccharine	0.1
Chlorhexidine gluconate	0.01
Dextranase	0.01

-continued

Formulation 5	
Ingredient	% w/v
Sodium monofluorophosphate	0.3
sCPP-ACP	5.0
Purified water	balance

pH adjusted to 5.5 with phosphoric acid

Formulation 6		
	% w/v	
Ingredient	1	2
Sorbitol	53.0	53.0
Silica (Zeodent 119)	20.0	20.0
Purified water	balance	balance
Sodium lauryl sulphate	4.0	4.0
sCPP-ACP	5.0	5.0
Sodium monofluorophosphate	—	0.3
Sodium dihydrogen phosphate	1.45	1.45
Flavour	1.0	1.0
Sodium carboxymethyl cellulose	0.75	0.75
Titanium dioxide (Rutile)	0.525	0.525
Xanthan gum	0.475	0.475
Sodium saccharin	0.350	0.350
Sodium fluoride	0.243	—

pH adjusted to 5-7 with phosphoric acid/NaOH

Formulation 7		
	% w/v	
Ingredient	1	2
Sorbitol (70% solution)	31.0	31.0
Purified water	balance	balance
Silica	17.0	17.0
Glycerol	8.0	8.0
Sodium lauryl sulphate	4.0	4.0
Polyethylene glycol 300	1.0	1.0
Sodium fluoride	0.243	—
Titanium dioxide (Rutile)	0.525	0.525
Xanthan gum	0.475	0.475
Sodium carboxymethyl cellulose	0.5	0.5
Sodium saccharine	0.286	0.286
Sodium acid pyrophosphate	2.4	2.4
Tetra sodium pyrophosphate	2.2	2.2
Flavour	1.0	1.0
sCPP-ACP	5.0	5.0
Sodium monofluorophosphate	—	0.3

pH adjusted to 5-7 with phosphoric acid/NaOH

EXAMPLE 6

Mouthwash Formulations

Formulation 1	
Ingredient	% w/v
Ethanol	10.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01

-continued

Formulation 1	
Ingredient	% w/v
Lauroyl diethanolamide	0.3
sCPP-ACP	5.0
Water	balance

pH adjusted to 5.5 using phosphoric acid/NaOH

Formulation 2	
Ingredient	% w/v
Gantrez S-97	2.5
Glycerine	10.0
Flavour oil	0.4
Chlorhexidine gluconate	0.01
Lauroyl diethanolamide	0.2
sCPP-ACP	5.0
Water	balance

pH adjusted to 5.5 using phosphoric acid/NaOH

EXAMPLE 7

Lozenge Formulation

Ingredient	% w/v
Sugar/sugar alcohol	75-80
Corn syrup	1-20
Flavour oil	1-2
sCPP-ACP	5.0
Mg stearate	1-5
Water	balance

pH adjusted to 5.5 using phosphoric acid/NaOH

EXAMPLE 8

Chewing Gum Formulation

Ingredient	% w/v
Gum base	30
Calcium carbonate	2.0
Crystalline sorbitol	53.0
Glycerine	0.5
Flavour oil	0.1
sCPP-ACP	5.0
Water	balance

pH adjusted to 5.5 using citric acid

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

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The invention claimed is:

1. A phosphopeptide or phosphoprotein (PP) stabilised amorphous calcium phosphate or amorphous calcium fluoride phosphate complex having a calcium ion content of about 30 moles or greater of calcium per mole of PP, wherein the phosphopeptide or phosphoprotein is a casein phosphopeptide or phosphoprotein or a tryptic digest thereof.

2. The complex according to claim **1**, wherein the calcium ion content is in the range of 30 to 50 moles of calcium per mole of PP.

3. An oral care formulation comprising the complex according to claim **1**.

4. A method for producing a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex having a calcium ion content greater than about 30 moles of calcium per mole of PP including the steps of:

(i) obtaining a solution including a PP-ACP and/or PP-ACFP complex; and

(ii) mixing with calcium and phosphate ions, while maintaining the pH of the solution at less than 7.

5. A formulation comprising a phosphopeptide or phosphoprotein (PP) stabilized ACP and/or ACFP complex and calcium phosphate, the formulation having a PP stabilized ACP and/or ACFP complex:calcium phosphate ratio of at least 1:1, wherein the phosphopeptide or phosphoprotein is a casein phosphopeptide or phosphoprotein or a tryptic digest thereof.

6. The formulation according to claim **5**, wherein the calcium phosphate is in the form of $CaHPO_4$.

7. An oral care formulation comprising the complex according to claim **6**.

8. A method for remineralizing a dental surface or subsurface including applying to the dental surface or subsurface a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP.

9. A method of treatment of one or more of dental caries, and dental erosion/corrosion, in animals including humans including applying to a dental surface or subsurface a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate or amorphous calcium fluoride phosphate complex having a calcium ion content greater than about 30 moles of calcium per mole of PP.

10. The complex according to claim **1**, wherein the calcium ion content is in the range of 30 to 100 moles of calcium per mole of PP.

11. The formulation according to claim **6**, wherein the PP stabilized ACP and/or ACFP complex:calcium phosphate ratio is 1:1-50.

12. The method according to claim **8**, wherein the PP is casein phosphopeptide (CPP).

13. The method according to claim **8**, wherein the calcium ion content is in the range of 30 to 50 moles of calcium per mole of PP.

14. The method according to claim **8**, wherein the calcium ion content is in the range of 30 to 100 moles of calcium per mole of PP.

15. The method according to claim **9**, wherein the PP is casein phosphopeptide (CPP).

16. The method according to claim **9**, wherein the calcium ion content is in the range of 30 to 50 moles of calcium per mole of PP.

17. The method according to claim **9**, wherein the calcium ion content is in the range of 30 to 100 moles of calcium per mole of PP.

18. The complex according to claim **1**, wherein the casein phosphopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3 and 4.

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